Reference: *Biol. Bull.* **191**: 70–75. (August, 1996)

Dynamic Encoding of Odors With Oscillating Neuronal Assemblies in the Locust Brain

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Abstract. The computational rules followed by the brain to encode complex, multidimensional stimuli such as natural odors are not well understood. In this review, we summarize results obtained in the olfactory system of an insect and present a hypothesis for odor representation in the brain. We propose that individual odors are represented by ensembles of neurons that are distributed both in space (the specific identities of the neurons forming an ensemble) and in time (the time at which each neuron participates in the ensemble response). In addition, we discuss the potential roles that periodic synchronization (oscillations) might play in this complex process.

Introduction

Odors in the natural world are usually complex blends of many volatile compounds. The percept that each natural fragrance evokes in us, however, is usually singular (*e.g.*, a rose, garlic, or a skunk). Our brains, therefore, probably form a unique internal representation of each specific blend, from which individual components (such as amylacetate, for example) must be difficult or impossible to segment. This specific odor representation must, in addition, be stable over time (odor memories are very long-lasting), and sufficiently inclusive to allow like odors (*e.g.*, roses of distinct varieties) to be "classified" as of the same sort.

A major challenge of neuroscientific research on olfaction is to understand the "computational" rules used by the brain to encode complex stimuli such as odors. Remarkable recent developments in vertebrate molecular biology allow us to understand some important aspects of mapping of odor-signals in the olfactory bulb (Buck and Axel, 1991; Vassar *et al.*, 1994; Sullivan *et al.*, 1995). These results complement physiological and imaging studies of odor processing indicating broad, distributed representation schemes (Cinelli *et al.*, 1995). Other recent fascinating results from studies of molluscan olfaction indicate that the olfactory nervous system of an invertebrate generates oscillations (Gelperin and Tank, 1990; Delaney *et al.*, 1994), a macroscopic functional feature similar to one long described in vertebrates (Adrian, 1942; Freeman, 1978; Satou, 1990). This result, combined with anatomical evidence that olfactory circuits in arthropods, molluscs, and vertebrates are built along very similar architectures, suggests that the computational rules used by olfactory systems may be similar (or conserved) across animal phyla and classes.

In this short review of our work, we summarize the dynamic and distributed scheme according to which we think odors are represented in the first and second olfactory relay stations of an insect brain. We address the issues of combinatorial coding, temporal representation, synchrony, and oscillations. The hypotheses presented here rely on recent electrophysiological, immunocytochemical, and morphological data from our laboratory, presented in Laurent and Naraghi (1994), Laurent and Davidowitz (1994), Leitch and Laurent (1996), and Laurent *et al.* (1996). We apologize to all authors whose

Received 30 November 1995; accepted 19 March 1996.

This paper was originally presented at a symposium titled *Finding Food: Neuroethological Aspects of Foraging*. The symposium was held at the University of Massachusetts, Amherst, from 6 to 8 October 1995.

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work is not cited and discussed here, and rather send readers to several excellent recent reviews on related and overlapping topics (Cinelli and Kauer, 1992; Freeman, 1992; Gray, 1994; Axel, 1995; Hildebrand, 1995; Hammer and Menzel, 1995; Singer and Gray, 1995).

Odor Representation in the Locust Olfactory System

In this section we review our main electrophysiological findings and derive from them a hypothesis for odor-coding, which might be applied to olfactory systems other than those of insects. When an odor (for example a pine fragrance) reaches the antenna of a locust (*Schistocerca americana*), a subset of the *ca.* 300 local and 850 projection neurons in the ipsilateral antennal lobe is activated. This subset may comprise 10% to 20% of the total complement of neurons in the antennal lobes. These activated neurons do not, however, all respond at the same time, or in the same way. We consider here two important and overlapping aspects of these response patterns: first, the slow and odor-specific temporal patterns of activity; second, the synchrony of firing and the oscillations of local field potential (Fig. 1).

Slow, odor-specific response patterns

When an odor is delivered to the antenna, some projection neurons fire for a few hundred milliseconds at the onset of the stimulus, while others fire only after a certain, consistent delay (from 10 to several hundred milliseconds). Other projection neurons yet may fire during 2 (or more) distinct epochs but remain silent in between. In other words, the ensemble of neurons that fire simultaneously (*i.e.*, within any 100-ms period of the ensemble response) changes as time progresses. This progressive change in the ensemble activated by an odor, how-

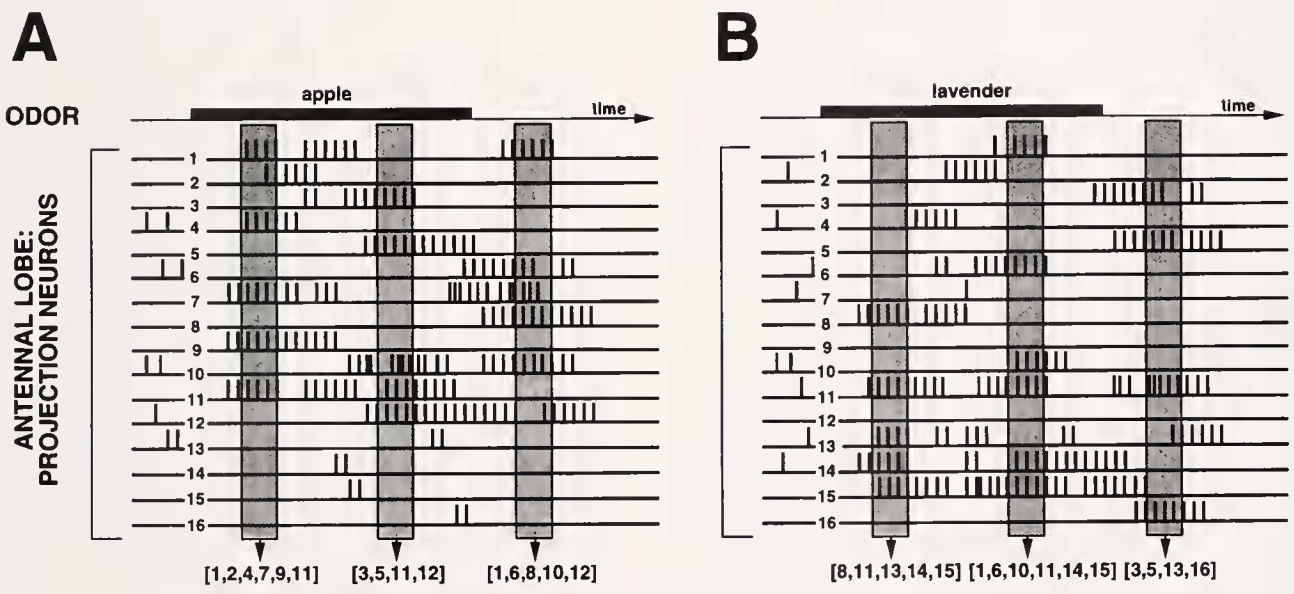

Figure 1. Distributed coding scheme for odors in the locust olfactory system. Each odor evokes spike activity in a subset of projection neurons in the antennal lobe (10%–15% of total complement of projection neurons). All responding neurons do not, however, respond at the same time. In the synthesized response represented in A, for example, neurons 1, 2, 4, 7, 9, and 11 respond together during epoch 1 (first gray window), whereas neurons 3, 5, 10, 11, and 12 respond together during the second highlighted epoch. Hence, the ensemble of neurons whose simultaneous activity represents the stimulus (here apple) changes progressively throughout the response. Superimposed on this distributed, evolving representation are synchronizing events, such that most of the active neurons in any one epoch fire together and periodically (20–30 Hz). The coherent firing of these subsets of neurons causes periodic activation of Kenyon cells in the mushroom body; this activation can be evidenced as local field potential oscillations with the same frequency. Because the membrane potential of individual Kenyon cells oscillates in response to presentation of the appropriate odor, it is hypothesized that the representation of the stimulus is refined in the mushroom body, by "selecting" inputs *only* from those projection neurons that are synchronized. In the example in A, for example, projection neuron 10 is not synchronized to the other active neurons (3, 5, 11, 12) during the second highlighted epoch. We propose, therefore, that the representation of the stimulus at this time is carried by neurons 3, 5, 11, and 12. The distributed representation obeys the same principles for each odor. In B, lavender is represented by a different evolving (overlapping) subset of projection neurons.

ever, is reliable over repeated presentations of the same odor, and is odor-specific. Indeed, presentation of a different odor (*e.g.*, a citrus fragrance) leads to the formation of a different (but partially overlapping) ensemble of activated projection neurons, with its own specific temporal features of activation. It appears, therefore, that a part of the odor representation relies on a specific spatial activity pattern (formed by the "physical" members of the odor-activated ensemble), as well as a specific temporal activity pattern (defined by the order in which the different neurons are recruited). Because both the spatial and temporal aspects of the response are reliable and odor-specific, we hypothesize that both must play a role in the encoding and "internal representation" of the odor signal.

Synchrony and 20–30 Hz oscillations

Intermeshed with the slow temporal activity patterns described above are more subtle aspects of neuronal activity: Antennal lobe neurons indeed often respond to odors not only by producing action potentials during specific time periods around the odor delivery, but also by locking these action potentials to a 20–30 Hz field potential oscillation that can be recorded extracellularly from the ipsilateral mushroom body. This field potential oscillation, which is synchronous over the entire mushroom body, is in fact the result of synchronous firing of a group of antennal lobe projection neurons, activated by an odor.

One first important point is that the seemingly uninterrupted 20–30 Hz field potential oscillations recorded in the mushroom body in response to an odor stimulus are caused by a sequential activation of groups of antennal lobe projection neurons. Indeed, because individual projection neurons generally respond only during a short period of the population response, each successive oscillation cycle is caused by the synchronous firing of a slightly different—and progressively evolving—subset of the activated projection neurons. If one could image the *ca.* 1100 antennal lobe neurons simultaneously and use the oscillation cycle duration (*ca.* 50 ms) as the "frame rate," one would see a specific succession of images created by the unfolding odor-evoked dynamical activity pattern.

A second important feature is that each projection neuron may not participate in the synchronized ensembles during the entirety of its response. Indeed, a projection neuron may produce action potentials during a significant period of the ensemble response but synchronize only a portion (*e.g.*, the first half, or the middle third) of these action potentials to the field potential. In other words, the fact that a projection neuron produces action potentials during an odor response does not necessarily mean that it participates in the ensemble oscillations during all (or any) of its response. The period during which a projection neuron synchronizes with the field potential oscillation (if it does) is, however, reliable and odor-specific. This indicates that it is not sufficient to consider only the number of action potentials produced by a projection neuron to understand its potential contribution to the ensemble representation. Rather, one must consider the precise timing of each action potential relative to the local field potential, *i.e.*, to the synchronous ensemble. Only a few of the action potentials contained in a response are generally phase-locked to the field potential and thus participate in the coherent activity reflected as oscillations in the mushroom body. The representation of an odor therefore appears to rely on an evolving population of synchronized projection neurons. Each projection neuron generally participates in the synchronized ensemble for a duration shorter than the ensemble oscillations, either because it is silent, or because it is not phase-locked during some of the ensemble response.

A third important feature of these oscillations relates to the phase of firing of individual projection neurons. When a projection neuron synchronizes to the local field potential, each spike occurs at a relatively precise phase (relative to the corresponding cycle of the field potential) that appears, so far, to be independent of the nature and concentration of the odor. Similarly, odor- or concentration-specific phase sequences that would indicate a coding scheme using phases or delays (Von der Malsburg and Schneider, 1986; Hopfield, 1995) have not been observed.

Where are these oscillations (or rather, the synchronization leading to the oscillations) generated? Ablation experiments showed that the calyx of the mushroom body is not necessary for the production of 20–30 Hz oscillatory response patterns in individual local and projection neurons in the antennal lobes. This suggests that the oscillations originate in the antennal lobes. Experiments in progress in which a GABA receptor antagonist is injected in various brain loci indicate that synchrony is abolished when inhibition is blocked in the antennal lobe but not when it is blocked in the mushroom body (MacLeod and Laurent, 1995). These observations are in agreement with ones made with the vertebrate olfactory system, showing that communication between olfactory bulb and piriform cortex is not required for the generation of odor-evoked theta (5–10 Hz) and gamma (30–60 Hz) oscillations in the olfactory bulb (Gray and Skinner, 1988).

To summarize, these data suggest that odors are represented by dynamic ensembles of neurons, and that the

oscillations evoked by odor presentation act, at least in part, as a "carrier wave" for a message distributed both among neurons (spatial representation) and in time (temporal representation). This message is "encoded" in the antennal lobe and sent to the mushroom body, where it may lead to a specific associative pattern representing the memory of this odor. The code is thus combinatorial, in that each odor is represented by a specific ensemble, and each neuron on its own can convey to the experimental observer only a little information about the identity of the stimulus.

Why Oscillations?

Our description of oscillations in the olfactory system is, of course, not the first. Odor-induced oscillations were first described in mammals more than 50 years ago (Adrian, 1942; Gray and Skinner, 1988), and were later observed in fish (see Satou, 1990) and molluscs (Gelperin and Tank, 1990; Delaney *et al.*, 1994). Our findings of oscillations in the locust olfactory brain and more recently in the cockroach and honey-bee brains (Stopfer and Laurent, 1995) add to the notion that processing in olfactory systems may use the same fundamental principles (or algorithms) across most animal phyla and classes (Hildebrand, 1995). But why oscillate at all? Although the existence of oscillations does not, in our minds, constitute the core of the hypothesized encoding scheme described above, it is a conspicuous (one might even sometimes argue distracting) component of it. For this reason, we will speculate here on why olfactory systems might oscillate.

One first and obvious possibility is that oscillations by themselves serve no particular purpose, but rather result simply from the functional architecture of olfactory networks. Oscillations, in this scheme, are only a by-product of processing by circuits built with prominent negative feedback loops (local-projection neuron interconnections). This hypothesis is plausible, given the similarity of microarchitecture of the olfactory circuits in the primary olfactory relays of insects, crustaceans, molluscs, and vertebrates. It is functionally rather uninteresting, however, because it assigns no functional purpose to neuronal synchronization.

A second possibility relates to the issue of learning. Maybe the ultimate goal of this part of the brain is to exploit synchronous activity patterns to form memories of the stimuli, using coincidence-dependent learning rules (*e.g.*, Hebbian learning rule; see for example Bourne and Nicoll, 1993). Indeed, it is important to realize that oscillations are the result of synchronization of large numbers of neurons, but that synchronization *per se* does not require oscillations (Singer and Gray, 1995)! One might indeed imagine nonperiodic synchronized activity patterns that would lead to nonoscillatory extracellular field potential activity. The problem is that, given the nature of biophysical and physiological systems, it is much easier to generate periodic than nonperiodic synchronous patterns. The existence of stimulus-induced oscillations might, in this context, therefore be a by-product of the "need" to produce synchrony for memory formation. Oscillations would therefore only be "useful" in the sense that they underlie coincident activity, with a "learning purpose." This hypothesis is more tempting (or pleasing), because it starts placing neuronal architecture, activity, and synaptic physiology into a coherent whole, and one that seems ultimately required for olfaction.

A third hypothesis is that setting up oscillations *de facto* creates a new variable—phase—with which to encode stimuli. Indeed, the existence of oscillations defines a periodic "clock" signal, in relation to which the timing of individual action potentials can be measured (Von der Malsburg and Schneider, 1986). Many theoretical arguments have been built that favor such a mechanism for purposes as diverse as segmentation (the separation of stimulus features such as object from background) in vision or hearing (Von der Malsburg and Schneider, 1986) or concentration-independent odor perception in olfaction (Hopfield, 1995). At present, however, the available data do not support these hypotheses, for only occasionally has phase been observed to vary, and to vary reliably, over repeated stimulus presentations (rat hippocampal place cells; O'Keefe and Recce, 1993). In most cases in which oscillations have been described, the phase of individual action potentials relative to the oscillations has been shown to be either remarkably consistent (under certain stimulus conditions) or inconsistently variable (under other stimulus conditions). To caricature these observations, it seems that the phase of spikes relative to the population (average) signal is either a constant (to a degree) or *unpredictably* variable. In other words, no consistent phase signature appears to be suggested by the available data. We therefore do not have any data supporting this third hypothesis at present.

A fourth hypothesis relates to a problem posed by distributed, combinatorial neuronal representations of sensory stimuli. If a stimulus (*e.g.*, an odor) is represented by an ensemble of neurons (rather than by one highly specific neuron, for instance), this distributed representation can be formalized as a vector sum of all the vectors contributed by each participating neuron (Georgopoulos, 1995). In this representation, the space where stimuli lie has as many dimensions as there are component neurons (about 850 here), and each odor can be represented by either a point (if the ensemble is static) or a

trajectory (if the ensemble is dynamic) within that space. The contribution of each neuron (*i.e.*, the length of the vector contributed by each neuron) is therefore measured by the number of spikes it provides to the ensemble. This, naturally, depends on the time over which spikes are counted. If the stimulus, or its representation, is dynamic, one needs a clock signal to update this population vector as the stimulus, and the response to it, both unfold. The oscillations may provide this clock signal, with the following added advantage: We observed that, if a neuron synchronizes to the field potential, it usually spikes only once per oscillation cycle. In other words, the contribution of each vector to the population vector is 0 (no spike or an unsynchronized spike) or 1 (one synchronized spike) at each oscillation cycle. A possible advantage is that the trajectory of the population vector is confined to the edges of an 850-dimension cube instead of occupying an infinitely large space. In this scheme, therefore, the oscillations would define the clock according to which the vector direction is updated, and simultaneously limit the possible length of this vector and of its trajectory. This type of reduction of the "coding space" might be very important in the design of an optimal memory system, whose role is to store new, and recognize old, distributed odor representations.

A fifth plausible hypothesis is that oscillations filter unwanted action potentials. In the case of the insect olfactory system, this would provide the means to build a first population odor representation in the antennal lobe, using a large subset of the available neurons (10%–20% of them, as suggested by the data), and to form a sparser representation, more convenient for memory storage, in the mushroom body, the second olfactory neuropil, where storage may be implemented (Hammer and Menzel, 1995; Davis, 1993). In this scheme, the Kenyon cells of the mushroom body would, using dendritic voltage-gated nonlinearities for example (see Laurent and Naraghi, 1994), associate inputs only from "perfectly" synchronized neurons, eliminating the contribution of ill-timed action potentials—and hence the contribution of the neurons producing these ill-timed action potentials. This method might thus transform, as well as "trim," the odor representation, making it less likely to saturate the memory capacity of the mushroom body and to lead to large overlaps and thus over-generalization.

Conclusions

Most of these hypotheses are of course nonexclusive and compatible with each other. They are also only some of many that space limitations (and limited imagination) prevent us from formulating here. The experimental problems facing us now are formidable. For any one of these hypotheses to be proven valid, it will have to be tested using a combination of refined physiological, behavioral, and psychophysical techniques. For example, one will want to ask the animal: "Did you smell and recognize odor X?" in conditions where firing synchrony, but not the identity of the firing neurons, is selectively disrupted. Because the tools for such experiments are, unfortunately, still undefined, this remains one of the most challenging tasks for neuroethologists. Our present findings clearly indicate that stimulus-specific temporal patterns of neuronal activity can be evoked in the brain, strongly suggesting that temporal codes (whether they are carried by a coherent oscillation or not) have a role to play in brain function. Whether this role is important, and what it might be, remains to be determined.

Acknowledgments

This work was supported by an NSF–Presidential Faculty Fellow and a McKnight Foundation Scholarship to Gilles Laurent.

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